COMPARISON OF SURFACE DIAMETERS AND DISSECTED DIAMETERS OF BOVINE OVARIAN FOLLICLES[1]

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ABSTRACT

Comparisons were made between diameters of 54 bovine follicles greater than 5.9 mm from 32 pairs of ovaries measured on the ovarian surface and diameters of the same follicles subsequently dissected from the ovaries. Seventy-eight percent of follicles measured on the ovarian surface were within 1.9 mm of the size measured after dissection. The remaining 22% of follicles measured on the surface had diameters recorded that were 2.0 to 3.9 mm different than their diameter after dissection. Surface diameter tended to underestimate dissected diameter for small follicles (<8.0 mm) and to overestimate dissected diameter for large (⩾ 12.0 mm) follicles. The correlation coefficient between surface and dissected follicular diameters was .83. We conclude that measuring the diameter of the largest follicles on the ovarian surface and after dissection yield approximately equivalent results.

(Key Words: Ovaries, Graafian Follicles, Cattle.)

Introduction

To assess ovarian follicular growth in cattle, many researchers have relied on measuring changes in either: 1) the size of the largest follicle per ovary (or pair of ovaries) or 2) the numbers of different-sized antral follicles within ovaries (Spicer and Echternkamp, 1986).

One common method used to assess size of bovine follicles is to measure diameter of follicles on the surface of ovaries (Dufour et al., 1972; Matton et al., 1981; Ireland and Roche, 1982, 1983a,b; Spicer et al., 1986a). Average diameters of the largest follicle on the ovarian surface in cattle vary from 10 to 14 mm during mid-luteal phase of the estrous cycle (Donaldson and Hansel, 1968; Ireland and Roche, 1983b) and from 14 to 19 mm just before ovulation (Donaldson and Hansel, 1968; Dufour et al., 1972; Ireland and Roche, 1982, 1983a). However, the accuracy of diameter measurements on the ovarian surface is questionable because much of a given follicle may be embedded within the ovary (Donaldson and Hansel, 1968). Presumably, diameters of dissected follicles should be a more accurate estimate of the actual diameter than surface diameter. However, surface measurements of diameter are much easier and faster to make than dissected diameters. In view of the increasing use of ovarian surface measurements to assess follicular growth dynamics in cattle (Matton et al., 1981; Ireland and Roche, 1982, 1983a,b; Spicer et al., 1986a), sheep (Dailey et al., 1982; Driancourt and Cahill, 1984) and primates (Clark et al., 1979; Kerin et al., 1981), it seems important to compare both diameter measurements. Therefore, the present study was conducted to compare quantitatively the diameters of follicles measured on the surface of ovaries with diameters of the same follicles subsequently dissected from the ovaries. We also related concentrations of steroids in fluid of follicles to the difference in surface and dissected diameters.

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Materials and Methods

Over a 3-wk period, 32 pluriparous, anovulatory lactating beef cows were ovariectomized as previously described (Spicer et al., 1986a). Immediately after ovariectomy, ovaries were placed on ice, and the diameters (long and short axes averaged) of the largest follicles that exceeded 5.9 mm on the surface of each ovary were measured using a vernier caliper. Efforts were made to identify the outer perimeter of each follicle, which included both clear and opaque surface areas of each follicle. This subjective method was used by the same individual throughout the study to assess surface diameter. These follicles were then dissected from the ovaries and excess stroma removed. Measurements of long and short axes for each dissected follicle were also averaged. Follicles >5.9 mm in diameter were selected for this study, since the relationship between follicular diameter and fluid volume is curvilinear for follicles <6 mm in diameter (Spicer and Echternkamp, 1986).

To compare the two diameter measurements, surface diameter was subtracted from dissected diameter. These differences were placed arbitrarily into four categories: ± 0 to .9 mm, ± 1.0 to 1.9 mm, ± 2.0 to 2.9 mm and ± 3.0 to 3.9 mm. The difference between the two measurements never exceeded ± 3.9 mm. Surface diameters were also categorized as: 6.0 to 7.9, 8.0 to 9.9, 10.0 to 11.9 and ≥12.0 mm, and the differences between surface and dissected diameters were calculated for each size category. To evaluate further the relationship between surface and dissected diameters, linear regression was conducted on these two variables.

Differences among means were evaluated by Student's t-test (Gill, 1978). Data on proportions of follicles in various groups were analyzed by chi-square (Gill, 1978).

Results and Discussion

Results in table 1 indicate that 41% of all follicles measured had surface and dissected diameters that differed by less than 1.0 mm, and that 37% differed by 1.0 to 1.9 mm. Thus, 78% of follicles measured on the surface were within 2.0 mm of its dissected diameter. The remaining 22% of follicles measured differed from 2.0 to 3.9 mm. Previously, Donaldson and Hansel (1968) found that 52% of follicles >5 mm measured on the surface were within 2 mm of the follicular diameter determined histologically. However, 27% of follicles measured by Donaldson and Hansel (1968) differed by more than 3 mm. This compares with only 5.5% in the present study. The discrepancies in results between these two studies can be explained, in part, by the different methods used to measure surface diameters. Donaldson and Hansel (1968) measured only the diameter of the translucent area of the follicle, while our measurements included both translucent and opaque areas. This also explains why all of the surface measurements made by Donaldson and Hansel (1968) were smaller than the follicular diameter determined histologically. We found that equal percentages of follicles were identified as smaller or larger than the diameter measured after dissection when measured on the ovarian surface (table 1). Thus, the conclusion stated by Donaldson and Hansel (1968) that "the

TABLE 1. FREQUENCY DISTRIBUTION OF DIAMETER MEASUREMENTS OF OVARIAN FOLLICLES CATEGORIZED BY THE DIFFERENCE BETWEEN DISSECTED DIAMETER AND SURFACE DIAMETER

Size difference, mm	% of total[a]		
	− Difference	+ Difference	Total
0[b]			5.6
.1– .9	20.4 (11)	14.8 (8)	35.2
1.0–1.9	20.4 (11)	16.7 (9)	37.0
2.0–2.9	5.6 (3)	11.1 (6)	16.7
3.0–3.9	1.8 (1)	3.7 (2)	5.5
Total	48.2 (26)	46.3 (25)	100

[a]No significant differences between + or − (chi-square, P>.10); (n) = number of follicles; − = surface diameter larger than dissected diameter; + = surface diameter smaller than dissected diameter.

[b]Three of 54 follicles had identical surface and dissected diameters.

technique of estimating maximum follicle diameter by diameter of the translucent follicle wall visible on the outside of the ovary has severe limitations" remains valid and does not conflict with our findings. However, since there is no "standard" with which to compare measurements, the relative accuracy of the various methods for assessing follicular diameter, i.e., histological evaluation, measurements of follicles on the ovarian surface, or measurements of dissected follicles, is not known. Certainly, it is easier to assess diameter of dissected follicles than to use histological preparations of the whole ovary. Moreover, surface diameters seem to be as good a method of diameter assessment as dissected diameter, since they were highly correlated (r = .83, figure 1). Transformation of the data to natural log did not improve the correlation coefficient (r = .81). Average diameter of all follicles (n = 54) was 10.1 ± 1.0 mm measured on the ovarian surface and 10.2 ± .9 mm measured after dissection. Each of the three techniques for assessing follicular diameter has shortcomings. During histological preparation of ovaries, length shrinkage due to tissue dehydration (estimated at 12.7%) can occur (Block, 1951), and only one plane (the cross-sectional plane) is readily available to quantify diameter.

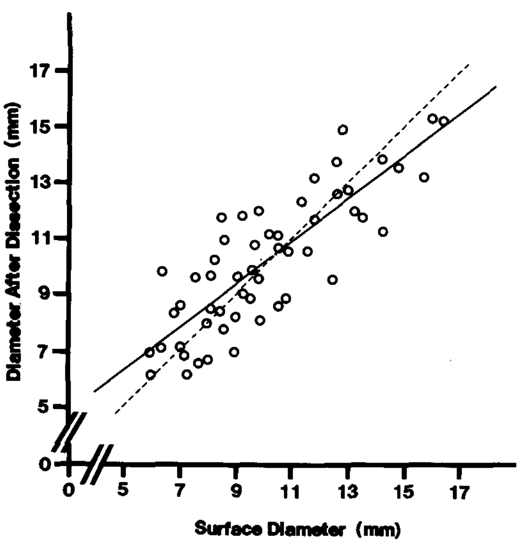

Figure 1. Relationship between diameter of follicles measured on the ovarian surface and diameter of the same follicles subsequently dissected from the ovary. A total of 54 follicles from 32 pairs of ovaries was compared. Simple correlation coefficient of actual data, r = .83 (P<.001). Fitted line (—): Y = .775X + 2.572. Theoretical line, (----): r = 1.0, Y = 1.0X + 0.

Diameter of the plane perpendicular to the plane of sectioning can be obtained by multiplying the width of sections by the number of these equidistant sections in which the follicle could be seen (Rajakoski, 1960). A similar procedure has been used to obtain diameter measurements for corpora lutea in guinea pigs (Rowlands, 1956). Physical restriction to follicular diameter imposed by the ovarian stroma is removed after dissection of follicles. Finally, surface diameters may be inaccurate because proportions of a follicle protruding from the ovarian surface may vary.

Results of the present study (table 2, figure 1) also indicate that surface diameter tends to underestimate dissected diameter for small follicles (<8.0 mm) and to overestimate dissected diameters for large follicles (⩾12.0 mm). Support for this conclusion is obtained when evaluating data of Ireland et al. (1979), who classified follicles into three groups based on follicular fluid volume measurements: small (5 to 100 μl), medium (101 to 400 μl), and large (>400 μl). Using an equation (relating follicular volume to dissected diameter) recently reported by us (Spicer and Echternkamp, 1986; log (diameter) = .266 × [log (volume)] + .267), one can convert follicular volumes reported by Ireland et al. (1979) to dissected diameters: calculated diameters would be 2.8 to 6.3 mm for small, 6.3 to 9.1 mm for medium and >9.1 mm for large. If follicular diameters were estimated from fluid volumes of Ireland et al. (1979) using the equation for volume of a sphere ($V = 4/3\pi r^3$), diameters would be 2.1 to 5.8 mm for small, 5.8 to 9.1 mm for medium and >9.1 mm for large follicles. Coinciding with the three follicular groups in Ireland et al. (1979) were surface diameters of small (<3 mm), medium (3 to 9 mm) and large (⩾10 mm). Thus, regardless of the method of calculation, diameters of small follicles in Ireland et al. (1979) were underestimated by over 3 mm using surface diameters, suggesting that diameters of dissected follicles may be more precise than surface diameters. This suggestion assumes that aspirated fluid volume of a follicle is the most precise measure of its size. Shortcomings of this assumption include the possibility that not all fluid may be removed from follicles, and granulosa cells aspirated with fluid may increase measured follicular volume. Nonetheless, diameters of small follicles calculated from the formula for spherical volume were .5 to .7 mm smaller than those calculated using the equation

TABLE 2. DIFFERENCES BETWEEN DISSECTED AND SURFACE DIAMETERS OF FOLLICLES AS AFFECTED BY SIZE OF THE FOLLICLE

Variable	Surface diameter, mm			
	6.0–7.9	8.0–9.9	10.0–11.9	12.0–16.9
n[a]	11	20	10	13
Avg surface diameter, mm	6.8 ± .2	8.9 ± .1	11.0 ± .2	14.0 ± .4
Avg size difference, mm[b]	.77[c]	.43[cd]	.12[d]	.42[d]

[a]n = number of follicles.

[b]Size difference = difference between dissected diameter and surface diameter; + = dissected diameter > surface diameter; − = dissected diameter < surface diameter. Pooled SE = ± .38.

[c,d]Differences without a common superscript differ ($P<.05$).

from Spicer and Echternkamp (1986). Two explanations for these differences in calculated diameters exist. First, dissected follicles typically had oval perimeters, thus not fitting the geometric restrictions of a sphere. Second, external diameters of dissected follicles would include several cell layers; thus, for a given follicular fluid volume (assuming a spherical shape), dissected diameter would be greater than the theoretical diameter calculated using follicular fluid volumes.

Finally, we hypothesized that growing antral follicles will be more likely to protrude from the ovarian surface, whereas follicles undergoing regression may have a greater proportion of their surface embedded within the ovary. Thus, surface and dissected diameters would be more similar for growing than regressing follicles. Since growing antral follicles in cattle produce more estradiol than progesterone (Ireland and Roche, 1982, 1983a,b; Bellin and Ax, 1984; Bushmeyer et al., 1985). we asked the question: can the difference between dissected and surface diameters be related to the follicles steroidogenic capability? To answer this question we categorized the follicles as either estrogen-active (concentrations of estradiol > progesterone in fluid; a modification of Ireland and Roche, 1982) or estrogen-inactive (concentrations of progesterone > estradiol in fluid). These are the same follicles in which steroid concentrations have been reported previously (Spicer et al., 1986b). The average difference between dissected and surface diameters (.1 ± .3 mm) was similar between follicles classified as estrogen-active and estrogen-inactive, indicating that the steroidogenic capacity of large follicles is not related to the difference in diameter measurements.

In summary, ovarian surface and dissected diameters of follicles were significantly correlated. However, there was a tendency for surface measurements to underestimate the size of dissected follicles 6 to 8 mm in diameter and to overestimate size of dissected follicles ⩾12.0 mm. We suggest that if precise follicular size estimates are desired within a short period of time, follicular size should be estimated by measuring follicular fluid volume (Spicer and Echternkamp, 1986). However, if only approximate size (± 3 mm) is desired, surface diameters are acceptable.

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